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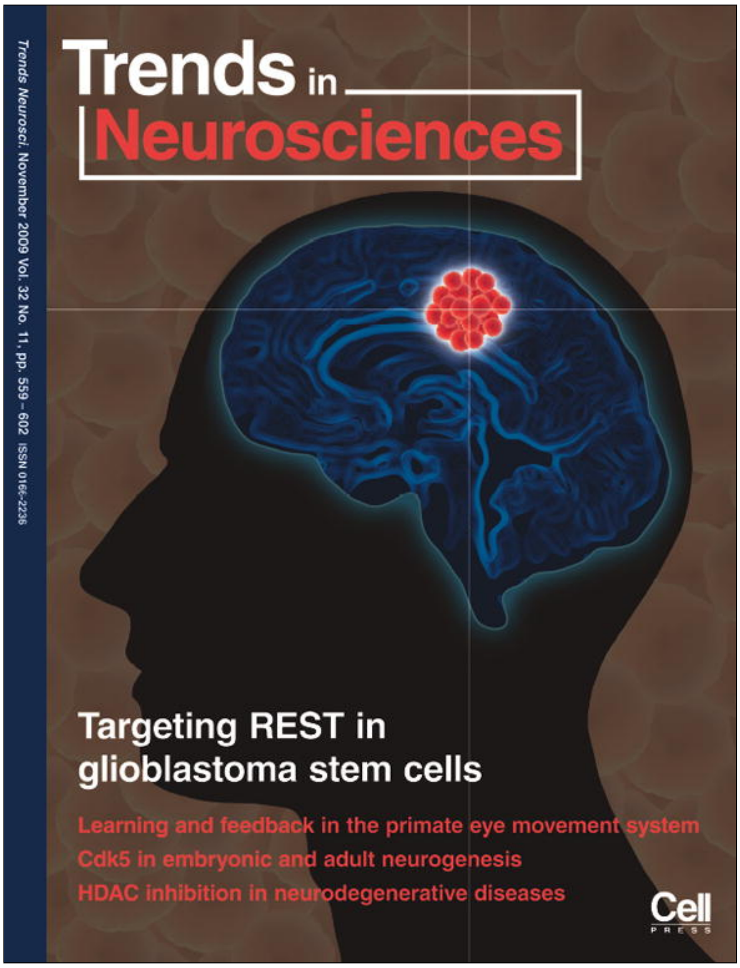

Trends Neurosci. November 2009 Vol. 32 No. 11, pp. 559–602 ISSN 0166-2236

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Review

Multiple roles of HDAC inhibition in neurodegenerative conditions

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Histone deacetylases (HDACs) play a key role in homeostasis of protein acetylation in histones and other proteins and in regulating fundamental cellular activities such as transcription. A wide range of brain disorders are associated with imbalances in protein acetylation levels and transcriptional dysfunctions. Treatment with various HDAC inhibitors can correct these deficiencies and has emerged as a promising new strategy for therapeutic intervention in neurodegenerative disease. Here, we review and discuss intriguing recent developments in the use of HDAC inhibitors to combat neurodegenerative conditions in cellular and disease models. HDAC inhibitors have neuroprotective, neurotrophic and anti-inflammatory properties; improvements in neurological performance, learning/memory and other disease phenotypes are frequently seen in these models. We discuss the targets and mechanisms underlying these effects of HDAC inhibition and comment on the potential for some HDAC inhibitors to prove clinically effective in the treatment of neurodegenerative disorders.

Introduction

Acetylation and deacetylation of histone proteins associated with chromatin plays a pivotal role in the epigenetic regulation of transcription and other functions in cells, including neurons [1–4]. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) catalyze the acetylation and deacetylation, respectively, of histone proteins at Lys (K) residues. The interplay between HATs and HDACs alters the net balance of histone acetylation levels, thereby remodeling chromatin structure (Figure 1). In general, an increase in protein acetylation at histone-tails results in a more open and relaxed chromatin conformation, thus facilitating transcription factor interaction with specific gene promoters and activating gene expression. HDACs often function as a component of the transcriptional repressor complex to silence gene expression and induce chromatin compaction through histone protein deacetylation. Accordingly, HDAC inhibition shifts the balance towards enhanced histone acetylation, chromatin relaxation and gene expression.

Imbalance between the activities of HATs and HDACs could lead to disease states. For example, mutation and loss of activity of the HAT, cyclic AMP response element binding protein (CREB)-binding protein (CBP), is causative for Rubinstein–Taybi syndrome, a developmental disorder characterized by mental retardation [5]. In addition to histones, HATs and HDACs also use a number of non-histone proteins as their substrates, notably tubulin and transcription factors such as the tumor suppressor p53, Sp1, Smad7, CREB, the pleiotropic transcription factor NF-κB, and signal transducers and activators of transcription-1 (STAT-1) (reviewed in Refs. [4,6]). In this article we first briefly describe the classification and isoforms of HDACs and the properties of a number of isoform-nonselective and more selective HDAC inhibitors. We then review the current research using various HDAC inhibitors in cellular and animal models of neurodegenerative diseases. The beneficial effects and potential caveats of these studies are discussed. Finally, proposed future directions are addressed.

HDACs and HDAC inhibitors

HDAC enzymes are evolutionarily conserved among many species. In humans, HDAC enzymes can be divided into four major classes based on their homology to yeast HDACs (reviewed in Refs. [7,8]). Class I HDACs include HDAC1, 2, 3 and 8; these are related to the yeast enzyme Rpd3. Class II HDACs include HDAC4, 5, 6, 7, 9 and 10, and are related to the yeast protein HDA1; class II HDACs are further divided into two subclasses – IIa (HDAC4, 5, 7 and 9) and IIb (HDAC6 and 10) – according to their structural similarities. Class I and II HDACs have been most extensively investigated for their roles in the central nervous system (CNS). Class III HDACs show dependence of nicotinamide adenine dinucleotide (NAD^+) and are referred to as sirtuins owing to their homology to the yeast HDAC Sir2. This class includes SIRT1–SIRT7 [9]. HDAC11, the most recently identified isoform, is a class IV HDAC due to its distinct structure [10]. Class I, II, and IV are zinc-dependent enzymes.

A variety of isoform-nonselective and selective HDAC inhibitors have been developed, both synthetically derived and from natural sources (reviewed in Ref. [6]). Among the relatively nonselective HDAC inhibitors, trichostatin A (TSA) and suberoylanilide hydroxamic acid (vorinostat, also known as SAHA) inhibit most zinc-dependent HDACs including HDAC6, and are permeable to the blood–brain barrier (BBB). The hydroxamate moiety of these compounds appears to bind the zinc ion at the HDAC active site to inactivate the enzyme. Sodium butyrate and 4-phenylbutyrate are fatty acid derivatives that inhibit most class I and II HDACs. However, butyrate does not appear to inhibit HDAC6 because the acetylation levels of

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0166-2236/$ – see front matter. Published by Elsevier Ltd. doi:10.1016/j.tins.2009.06.002 Available online 21 September 2009

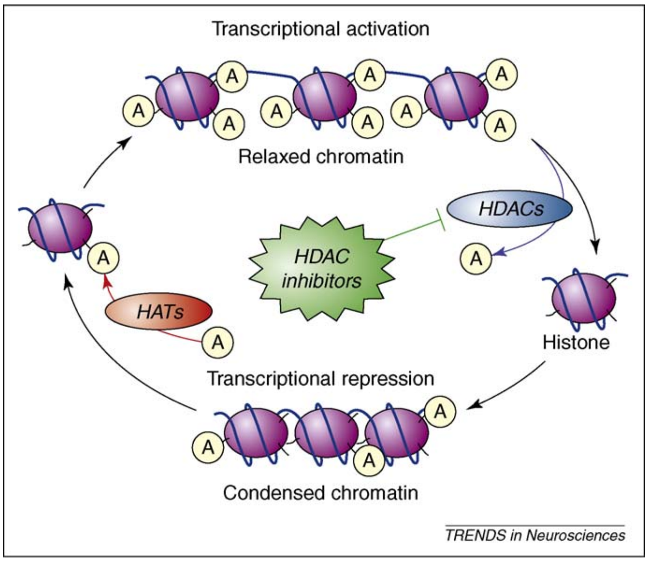
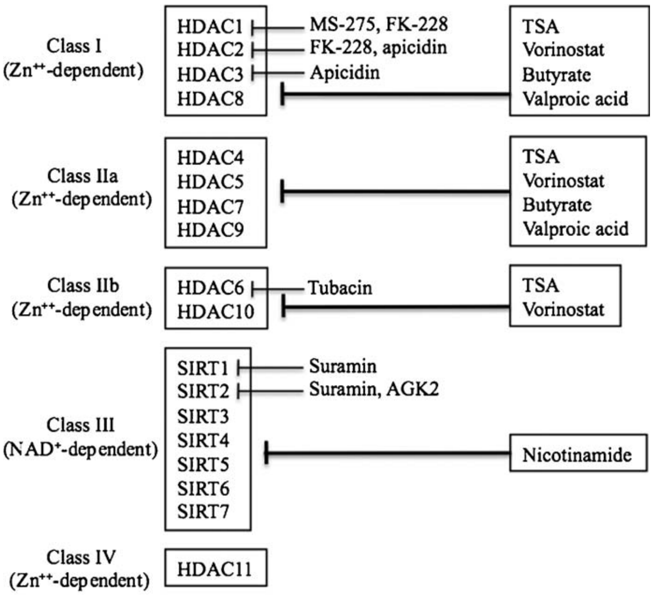


Figure 1. Effects of HDAC inhibitors on chromatin remodeling. Levels of histone acetylation at Lys residues on histone-tails are determined by interplays of acetylation and deacetylation catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. Inhibition of HDACs by HDAC inhibitors results in a net increase in histone acetylation levels and a more open, relaxed chromatin conformation that favors transcriptional activation. By contrast, chromatin with a compact conformation is transcriptionally inactive. Ⓐ, acetylated Lys residues of histone-tail proteins.

Table 1. HDAC isoforms and isoform-specific and nonspecific HDAC inhibitors[a]



Class I (Zn^{++}-dependent)	HDAC1	MS-275, FK-228	TSA Vorinostat Butyrate Valproic acid
	HDAC2	FK-228, apicidin	
	HDAC3	Apicidin	
	HDAC8		
Class IIa (Zn^{++}-dependent)	HDAC4 HDAC5 HDAC7 HDAC9		TSA Vorinostat Butyrate Valproic acid
Class IIb (Zn^{++}-dependent)	HDAC6	Tubacin	TSA Vorinostat
	HDAC10		
Class III (NAD$^+$-dependent)	SIRT1	Suramin	Nicotinamide
	SIRT2	Suramin, AGK2	
	SIRT3 SIRT4 SIRT5 SIRT6 SIRT7		
Class IV (Zn^{++}-dependent)	HDAC11		

[a]Detailed information and reference citations are described in the text.

α-tubulin, a substrate of HDAC6 [11], are unaffected by sodium butyrate treatment. Valproic acid, a fatty acid derivative with mood-stabilizing and anticonvulsant properties, is another HDAC inhibitor that binds to the active site of the enzymes [12,13]; valproic acid inhibits class I and class IIa HDACs, but not class IIb [14]. Butyrates and valproic acid are also known to readily cross the BBB (reviewed in Ref. [15]).

Advances have been made to design more selective HDAC inhibitors. MS-275, a synthetic benzamide derivative, preferentially inhibits HDAC1, compared with HDAC2, 3, and 9, and has little or no activity against HDAC4, 6, 7, and 8 [16]. This drug also passes the BBB easily, can be administered orally, and appears to produce no severe side effects. Apicidin, a cyclic tetrapeptide, inhibits HDAC2 and 3 in the low nanomolar range and HDAC8 in the high nanomolar range, but does not affect HDAC1 or class II HDACs [16]. Romidepsin (FK-228), another cyclic tetrapeptide, also potently inhibits HDAC1 and 2 [6]. Tubacin is a catalytic domain-targeting small molecule inhibitor showing high selectivity for HDAC6 and for the deacetylation of α-tubulin, a microtubule component [17]. However, tubacin only has about 4-fold selectivity over HDAC1 and HDAC4 [18]. Suramin, a symmetric polyanionic naphthylurea, and its structural analogs, inhibit human NAD$^+$-dependent class III SIRT1 and SIRT2 activity [19]. Nicotinamide, also known as niacinamide, is a precursor of NAD$^+$ and a competitive class III HDAC inhibitor that can be given orally [20]. It should be noted that different studies have reported widely varying IC$_{50}$ values for the inhibition of specific HDAC isoforms by a given inhibitor. It is recommended that K$_i$ measurements of HDAC inhibitors should be performed in future investigations so as to minimize discrepancies between studies. Table 1 lists the isoforms of these four classes of HDACs and their sensitivities to the key HDAC inhibitors discussed in this review.

Neuroprotection by HDAC inhibition in cellular models

HDAC inhibition has neuroprotective effects in both *in vivo* and *in vitro* models of brain disorders. One pioneering study noted that levels of the HATs CBP/p300 and histone protein acetylation were decreased during apoptosis induced by potassium deprivation of cultured primary cerebellar granule cells, and during signal activation of β-amyloid precursor protein (APP) in cultured primary cerebral cortical neurons from rodents [21]. Moreover, overexpression of CBP/p300 protected these neurons from proapoptotic insults. In cultured cortical neurons, Ryu and colleagues showed that treatment with TSA, sodium butyrate, or vorinostat protected against glutathione depletion-induced oxidative stress; neuroprotection involved acetylation and activation of the DNA binding activity of Sp1 [22]. However, it is well known that some HDAC inhibitors, such as TSA, have basal toxicity and prolonged treatment at high doses often induces neuronal death, so compromising their neuroprotective effects [23]. HDAC inhibitor-induced neurotoxicity could be partly due to 'derepression' of genes involved in apoptosis including *Bim* and *B-myb* [24]. In an effort to sidestep this issue, Langley and colleagues found that a two-hour pulse treatment with TSA sufficed to rescue cortical neurons from oxidative stress without obvious toxicity; protection was associated with transcriptional activation of the cell-cycle inhibitor p21$^{waf1/cipl}$ [25]. Notably, p21$^{waf1/cipl}$ is sufficient, but not necessary, for protection by HDAC inhibition, and its action appears to be independent of its ability to inhibit cell-cycle progression. A very recent study showed that class I/II HDAC inhibitors blocked Bax-dependent apoptosis of mouse cortical neurons by p53-dependent and -independent mechanisms [26]. This study identified Bax as a convergent target for these two distinct neuroprotective pathways.

Glutamate-induced excitotoxicity has been implicated in the pathophysiology of many neurodegenerative and neuropsychiatric diseases; these include stroke, Huntington's disease, amyotrophic lateral sclerosis, spinal cord and traumatic brain injury, cerebellar degeneration and possibly Alzheimer's disease, Parkinson's disease and mood disorders [27]. Notably, Chuang and colleagues showed that the HDAC inhibitor valproic acid protected against excitotoxicity in cultured primary neurons induced by exposure to glutamate [28] or SYM 2081 [29], a blocker of excitatory amino acid transporters and an agonist of low-affinity kainate receptors. In the latter study, the protective effects of valproic acid were mimicked by treatment with structurally similar and dissimilar HDAC inhibitors and were associated with decreased levels of excitotoxicity-induced accumulation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in neuronal nuclei, an effect apparently due to weakening of histone interactions by their hyperacetylation. Numerous studies have established that overexpression and nuclear translocation of GAPDH, a glycolytic enzyme traditionally thought to be a housekeeping gene, have proapoptotic roles in cellular and animal models of neurodegenerative conditions; the proapoptotic effects were independent of the glycolytic activity of GAPDH (reviewed in Ref. [30]). However, the detailed nuclear mechanisms underlying GAPDH-induced neuronal apoptosis remain obscure. Interestingly, Snyder, Sawa and colleagues recently reported that GAPDH is acetylated at K160 by interaction and activation of the HATs p300/CBP in the nucleus, resulting in acetylation and stabilization of proapoptotic proteins including p53 [31]. Although the exact role of GAPDH acetylation is undefined, their findings suggest that drugs that disrupt the nuclear GAPDH-p300/CBP complex may be developed as anti-apoptotic agents.

Leng and Chuang demonstrated that valproic acid, 4-phenylbutyrate, or TSA treatment protected against glutamate-induced, NMDA receptor-mediated excitotoxicity in cerebellar granule cells, with concomitant transcriptional activation and induction of α-synuclein, a presynaptic protein of unknown function [28]. Knockdown with α-synuclein siRNA or antisense oligonucleotides confirmed that overexpression of endogenous α-synuclein plays a neuroprotective role; this appears to involve upregulation of the cytoprotective protein B-cell lymphoma 2 (Bcl-2) but downregulation of Ube2n, a proapoptotic, ubiquitin-conjugating enzyme. A subsequent study showed that α-synuclein protected cerebellar granule cells from 6-hydroxydopamine-induced death [32]. It appears that α-synuclein is neuroprotective in the cytoplasm, but becomes neurotoxic once translocated to the nucleus, where it inhibits HAT activity [28,32,33]. A more recent study by Leng *et al.* found that, under certain experimental conditions, valproic acid and other class I and II HDAC inhibitors (e.g. sodium butyrate, 4-phenylbutyrate, and TSA) potentiated these neuroprotective effects against excitotoxicity when used in conjunction with lithium, another mood stabilizer with a robust neuroprotective profile [34]. This neuroprotective synergy was mediated, at least in part, by enhanced inhibition of glycogen synthase kinase-3 (GSK-3) so as to potentiate β-catenin–Lef–Tcf-dependent transcriptional activity, part of the Wnt signaling pathway.

Taken together, the evidence reviewed above suggests that HDAC inhibitors induce the expression of multiple downstream targets that might work collectively to elicit neuroprotective effects. Lending further support to this view, HDAC inhibitors increase the expression of neurotrophins, molecules that play prominent roles in neuronal development, synaptic plasticity, and neuronal survival. For instance, Yasuda *et al.* found that brain-derived neurotrophic factor (BDNF) was induced in rat cortical neurons by treatment with valproic acid, sodium butyrate, or TSA, an induction that involved activation of the BDNF promoter IV [35]. Transfection of siRNA specific for HDAC1 also activated BDNF promoter IV, suggesting a regulatory role for this HDAC isoform in BDNF expression. Hong and colleagues found that both BDNF and glial cell line-derived neurotrophic factor (GDNF) were induced by class I and II inhibitors in primary cultures of astrocytes from rat midbrain [36,37]. GDNF induction by HDAC inhibition in astrocytes is associated with histone H3 hyperacetylation in the promoter of the gene encoding GDNF, and contributes to trophic effects on midbrain dopaminergic neurons. In addition, emerging evidence supports the notion that HDAC inhibition in microglia plays a highly significant role in mediating anti-inflammatory effects. HDAC inhibitors robustly protected against dopaminergic neuronal death and neuroinflammation induced by exposure to lipopolysaccharide (LPS) [36,38]. Anti-inflammatory effects were characterized by the inhibition of LPS-induced microglial activation, TNF-α release, and nitric oxide production, and were at least partially mediated by triggering apoptosis of overactivated microglia through the disruption of mitochondrial membrane potential [39]. Likewise, sodium butyrate was shown to be anti-inflammatory in LPS-treated brain-derived primary microglia [40]. The anti-inflammatory effects of HDAC inhibitors were also found in an animal model of cerebral ischemia (see below). Taken together, the *in vitro* studies demonstrate that HDAC inhibitors exert their neuroprotective effects through multiple mechanisms and that, in addition to neurons, glia are also targets of HDAC inhibition and neuroprotection.

HDAC inhibition in animal models of neurodegenerative disorders

Stroke

Stroke, an acute neurological/neurodegenerative disease, is the third leading cause of death in the USA. Most stroke cases are caused by cerebral ischemia. In a middle cerebral artery occlusion (MCAO) stroke model, reduced bulk histone acetylation was found at Lys residues in the ischemic brain of rats or mice; these changes were restored by treatment with HDAC inhibitors, with a concomitant decrease in infarct volume [41–43]. In a rat MCAO model, Chuang and colleagues showed that post-insult treatment with valproic acid, sodium butyrate or TSA also improved behaviors [41,42]. The long-term behavioral benefits in sodium butyrate-treated MCAO rats were associated with enhanced neurogenesis in the ischemic brain; this was abolished by blocking BDNF–*TrkB* signaling pathways [44]. In addition, administration of 4-phenylbutyrate in mice subjected to hypoxia–ischemia protected against endoplasmic reticulum (ER) stress [45], evidenced by decreased eIF2α phosphorylation and expression of the eIF2α-regulated proapoptotic protein CHOP.

It is increasingly recognized that neuroinflammation plays a causative role in neurodegeneration following ischemic injury. Kim *et al.* demonstrated that post-insult treatment with valproic acid or sodium butyrate suppressed permanent MCAO-induced activation of microglia and monocytes/macrophages and proinflammatory iNOS and COX-2 overexpression [42]. Treatment with HDAC inhibitors also markedly inhibited ischemia-induced p53 overexpression and superinduced heat shock protein 70 (HSP70) in the ischemic brain [41–43]. It is likely that superinduction of endogenous HSP70 by HDAC inhibition contributes to these anti-inflammatory effects. In support, one recent study noted that HSP70 overexpression inactivated NF-κB by stabilizing a complex of HSP70–IκBα–NF–κB in a mouse MCAO model [46].

The expression of cytoskeletal proteins has also been implicated in neuroprotection by HDAC inhibition under ischemic conditions. For instance, HDAC inhibition upregulated gelsolin, a protein involved in actin filament organization, and by this route contributed to neuroprotection from ischemic brain injury [47,48]. In addition, valproic acid was neuroprotective in an intracerebral hemorrhagic model of stroke by HDAC inhibition and transcriptional activation, and displayed anti-inflammatory actions by down-regulating proinflammatory factors including Fas-L, IL-6, and MMP-9 [49]. The identity of the HDAC isoform(s) involved in HDAC inhibitor-mediated neuroprotection remains unclear. However, from the related cardiac field, it is noteworthy that knockdown of HDAC4 reduced infarct size following myocardial ischemia-induced reperfusion injury [50].

Huntington's disease (HD)

HD is an inherited, autosomal-dominant fatal neurodegenerative disease characterized anatomically by a predominant loss of striatal medium-sized spiny neurons and cortical neurons, and clinically by hyperkinetic involuntary movement, cognitive impairment and memory loss, as well as by psychosis and emotional deterioration. It is well known that the genetic mutation responsible for HD is an expansion of a CAG trinucleotide repeat encoding polyglutamine (polyQ) in the first exon of the huntingtin (*HTT*) gene. Transcriptional dysregulation plays a central role in the pathogenesis/pathophysiology of HD [51,52]. For example, HTT with an expanded polyQ repeat has been shown to interact with and impair neuroprotective transcription factors such as Sp1 and its co-activator $TAF_{II}130$, as well as HATs including CBP and p300/CBP associated factor CP/CAF (reviewed in Refs. [4,53]).

Treatment with vorinostat or TSA suppressed ongoing neuronal photoreceptor degeneration and reduced lethality in transgenic *Drosophila* expressing mutant HTT [54]. Complicating the picture, a subsequent study employing a *Caenorhabditis elegans* HD model demonstrated that knockdown of *hda-3* suppressed neurodegeneration in response to HTT-Q150 [55]. Conversely, deletion of one copy of *hda-1* enhanced polyQ neurotoxicity, and this was unaffected by *hda-3* loss of function. These results suggest that the two encoded HDAC isoforms act on different targets to induce opposite effects on polyQ toxicity. Another report showed that neurodegeneration was sensitive to the zinc-dependent fly HDAC Rpd3, whereas genetic or pharmacological blockade by nicotinamide of NAD^+-dependent class III HDAC Sir2 or Sirt2 was neuroprotective in a *Drosophila* HD model [56]. Additional neuroprotection was achieved when Rpd3 and Sir2 were simultaneously inhibited. These results suggest that, in addition to class I and II HDACs, class III HDACs are also potential targets in HD and other diseases where polyQ is central to pathogenesis. Importantly, a very recent report by Jeong and colleagues showed that HTT was acetylated at K444 by the HAT, CBP [57]. Enhanced K444 acetylation facilitated the trafficking of mutant HTT into autophagosomes for degradation and reduced the neurotoxicity of mutant HTT both in primary neuronal cultures and in a *C. elegans* HD model. These findings identify acetylation of HTT as a new mechanism for clearing accumulated HTT protein and suggest that increased HTT acetylation is a potential target for HDAC inhibition to elicit neuroprotective effects in HD.

R6/2 mice express an N-terminal portion of human HTT with 150 or more glutamine repeats and display an early onset disease phenotype. Hockly and colleagues showed in this model that vorinostat improved motor performance in a rotarod test but did not affect polyQ aggregation or downregulate the expression of the expanded *HTT* transgene [58]. Using the same model, Ferrante and colleagues reported that sodium butyrate treatment decreased the neurodegenerative phenotype and improved survival [59]. In R6/2 mice, hypoacetylation associated with gene expression downregulation and mRNA aberrations in affected brain regions was corrected by treatment with 4-phenylbutyrate [60] or with a novel pimelic diphenylamide HDAC inhibitor, $HDAC_i$ 4b [61]. Improvement of motor dysfunction, normalization of striatal atrophy, and prevention of brain weight loss were also observed using $HDAC_i$ 4b, a synthetic benzamide derivative with relatively low toxicity. Furthermore, post-symptomatic (at 11 weeks) chemotherapy with 4-phenylbutyrate prolonged lifespan and ameliorated brain anatomical deficits, but failed to improve rotarod performance in N171-82Q HD transgenic mice [62].

It has been suggested that the pathophysiology of HD is coupled intimately to deficiency of both BDNF and HSP70 in affected brain regions [63–65]. Because the expression of both BDNF and HSP70 is regulated by class I and II HDAC inhibitors, it is conceivable that restoring BDNF and HSP70 to their normal levels contributes to the beneficial effects of HDAC inhibition in various HD models. In this context, it is notable that vorinostat and TSA (but not 4-phenylbutyrate or MS-275) increase vesicular transport of BDNF by inhibiting HDAC6 in particular, thereby increasing tubulin acetylation and compensating for the transport deficit in HD [66]. However, there is a potential drawback because the role of HDAC6 in HD pathology is clearly complex – for example, HDAC6-dependent retrograde transport on microtubules is crucial for the autophagic degradation of aggregated HTT [67] and HDAC6 is therefore likely to be neuroprotective in this role. Further, expression of HDAC6 rescues polyQ-induced neurodegeneration associated with dysfunction of the ubiquitin–proteasome system in a fly model of spinobulbar muscular atrophy [68]. The dual roles of HDAC6 in neurodegeneration and neuroprotection complicate the application of this subtype-specific inhibition in treating polyQ-induced neurodegenerative diseases.

Amyotrophic lateral sclerosis (ALS)

ALS is an adult-onset neurodegenerative disease characterized by progressive loss of motor neurons in the brain, brain stem, and spinal cord; these lead to generalized weakness, muscle atrophy, paralysis, and eventual mortality within five years of disease onset. Most ALS cases occur sporadically, with only about 10% of patients being categorized as having a familial form. Of these, approximately 20% are attributed to gain-of-function mutations in the gene encoding Cu/Zn superoxide dismutase 1 (SOD1), a key antioxidant enzyme.

Mice expressing mutant Cu/Zn SOD1 exhibit ALS-like phenotypes, including the formation of intracellular aggregates of SOD1 in the brain and spinal cord, behavioral abnormalities, and premature death. Because transcriptional dysregulation may play a role in the pathophysiology of ALS, the effects of HDAC inhibitors have been examined in transgenic ALS mouse models. Using SOD1/G93A transgenic mice, Ryu and colleagues injected 4-phenylbutyrate starting before or shortly after symptom onset; this resulted in extended survival and improved pathological phenotypes [69]. The study also found that 4-phenylbutyrate treatment ameliorated hypoacetylation, upregulated Bcl-2, NF-κB, p50 and phospho-IκB, and downregulated cytochrome *c* and caspases in the spinal tissues of G93A mice. Using the same ALS transgenic mice, Ferrante and colleagues showed that combined treatment with phenylbutyrate and riluzole, the only FDA-approved drug for treating ALS, was more effective than either drug alone in increasing survival and improving pathological phenotypes [70]. Moreover, Petri and colleagues reported that co-treatment with 4-phenylbutyrate and AEOL 10150, a catalytic antioxidant, had a cumulative effect on survival time and also reduced markers of oxidative damage in the lumbar spinal cord [71] of the ALS mice; this suggests that multiple molecular mechanisms are involved in the pathophysiology of ALS. In support, microarray analysis of SOD1/G93A mice revealed changes *versus* controls in the expression of a large number of genes involved in the progression of motor neuron injury, including those involved in transcriptional and translational functions [72].

Valproic acid treatment of SOD1 mutant mice has had variable effects on disease symptom onset, duration, and survival. For instance, pre- or post-symptomatic valproic acid treatment in drinking water increased lifespan, but pre-symptomatic treatment had no effect on the onset of motor symptoms in G93A mice [73]. Rouaux and colleagues found that, in G86R SOD1 mutant mice, valproic acid injections maintained normal levels of histone acetylation, restored the loss of CBP and significantly suppressed the death of motor neurons, although it did not prolong survival [74]. More recently, Feng *et al.* reported that valproic acid treatment of G93A mice had small, but significant, beneficial effects on motor dysfunction onset, motor deficits, and survival time [75]. These inconsistent results could arise from differences in valproic acid dosing, treatment method and duration, the number of copies of the mutant *SOD1* gene, and/or the strain of transgenic ALS mice used. In the latest study, combined treatment with valproic acid and lithium produced greater and more consistent benefits than valproic acid alone in delaying the onset of disease symptoms, prolonging lifespan, and decreasing neurological deficits [75]. Valproic acid and lithium co-treatment was also more effective than either drug alone in enhancing Ser9 phosphorylation of GSK-3β in the brain and in lumbar spinal cord, suggesting enhanced inhibition of GSK-3 activity. These observations are reminiscent of the synergistic neuroprotective effects of combined valproic acid–lithium treatment in cultured neurons (see above). These and other studies highlight the potential effectiveness of combination treatments for ALS patients. A phase 2 study using sodium phenylbutyrate has been reported [76]. Following treatment for 20 weeks in 26 participants, phenylbutyrate was found to be safe and well-tolerated in a dose range of 9 to 21 g/day and increased blood histone acetylation levels.

Spinal muscular atrophy (SMA)

SMA is an autosomal-recessive inherited motor neuron disease caused by degeneration of α-motor neurons in the anterior horn of the spinal cord and is characterized by weakness and atrophy of voluntary muscles. The genetic basis of SMA, a leading hereditary cause of infant mortality, is homozygous deletion of the *SMN1* gene on chromosome 5q13 that encodes the full-length survival motor neuron protein. A related gene located within the same chromosomal locus, *SMN2*, is ubiquitously expressed but encodes an unstable SMN protein lacking C-terminal residues. Although SMA patients lacking *SMN1* carry at least one copy of *SMN2*, the amount of functional SMN protein produced by *SMN2* is insufficient to combat progressive motor neuron degeneration. Disease severity appears to be inversely correlated with *SMN2* gene copy number and SMN expression, suggesting that *SMN2* is a potential therapeutic target. A number of HDAC inhibitors increase *SMN2* mRNA and protein levels *in vitro,* including sodium butyrate [77], 4-phenylbutyrate [78], valproic acid [79–81], M344 [81–83], vorinostat [81,83], TSA [84] and romidepsin (FK-228) [83]. Interestingly, the *SMN2* promoter associates with HDAC1 and HDAC2, but not with HDAC3–5 [85], indicating that different HDAC isoforms play selective roles in regulating *SMN* gene expression.

Chang and colleagues provided the first evidence that administering sodium butyrate to a transgenic mouse model of SMA ($Smn1^{-/-}$ *SMN2*) increased SMN protein expression, combated clinical symptoms, and increased their lifespan [77]. Using a similar mouse model, Tsai and colleagues showed that oral administration of valproic acid in drinking water improved motor function, promoted motor-evoked potential and neuromuscular junction formation, and also suppressed spinal motor neuronal degeneration and muscular atrophy [86,87]. The beneficial effects of valproic acid were associated with elevated levels of SMN mRNA and protein in spinal tisues as well as increased levels of the anti-apoptotic proteins Bcl-2 and Bcl-X_L. The upregulation of Bcl-X_L probably contributes to valproic acid-induced neuroprotection because transgenic overexpression of Bcl-X_L also improved pathological features and lifespan in the mouse SMA model [88]. Avila and colleagues used repeated daily injections of TSA commencing after disease onset to show that HDAC inhibition activated spinal *SMN2* gene expression and improved motor pathology and survival in SMA-like mice [84]. BDNF mRNA levels in spinal cord and muscle of these mice were also markedly increased after TSA treatment.

A pilot trial using 4-phenylbutyrate increased SMN mRNA in the leukocytes of SMA patients and improved their functional performance scale [89,90]. Similarly, valproic acid treatment increased SMN mRNA and protein in leukocytes of SMA patients [91] and improved patients' muscle power [92,93]. It should be noted that the beneficial effects of valproic acid and butyrate in both preclinical and clinical studies of SMA are limited. Future SMA studies using other HDAC inhibitors, notably class III inhibitors, seem warranted. Combinatorial treatment with an HDAC inhibitor in conjunction with another drug exhibiting different actions would also be a rational approach.

Parkinson's disease (PD)

PD is a prevalent neurodegenerative disease characterized by a relatively selective loss of dopaminergic neurons, predominantly in the substantia nigra. Most cases of PD occur sporadically. An established model of PD involves treatment of mice with the classical dopaminergic toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Pioneering work by Beal and colleagues [94] showed in this model that administration of phenylbutyrate significantly attenuated both dopamine depletion and loss of substantia nigra neurons expressing the dopamine biosynthetic enzyme tyrosine hydroxylase. Using a related dopaminergic toxin, 1-methyl-4-phenylpyridinium (MPP^+), in an *in vitro* model of midbrain neuron–glia cocultures, Hong and colleagues found that MPP^+-induced death of dopaminergic neurons could be prevented by treatment with valproic acid, sodium butyrate, or TSA, as demonstrated by marked increases in dopamine uptake and the number of neurons staining positive for tyrosine hydroxylase [36,37]. As discussed above, these treatments also induce GDNF expression in midbrain astrocytes through HDAC inhibition. Notably, the use of GDNF gene delivery has been considered as a potential therapy for neurodegenerative diseases including PD [95]. Therefore, screening for HDAC inhibitors that can more robustly upregulate endogenous GDNF in the brain seems warranted for drug development in PD and other brain disorders.

Mutations of a number of targets, including the presynaptic protein α-synuclein, have been linked to familial forms of PD. One study reported that nuclear α-synuclein binds histones to inactivate HATs including CBP, p300 and P/CAF, causing histone hypoacetylation and apoptosis in human neuroblastoma cells; these toxic effects were potentiated by mutations affecting α-synuclein [33]. By contrast, cytoplasmic accumulation of α-synuclein is neuroprotective both in cultured cells and in a transgenic *Drosophila* model of PD. An α-synuclein mutation linked to PD promotes nuclear targeting and neurotoxicity in cultured cells. Treatment with sodium butyrate or vorinostat reduced neuronal death in response to α-synuclein overexpression both *in vivo* and *in vitro* [33]. More recently, Outeiro and colleagues reported that AGK2, a specific inhibitor of SIRT2, increased α-tubulin acetylation with the formation of large α-synuclein inclusions, and was able to rescue dopaminergic neurons both *in vitro* and in a *Drosophila* PD model [96]. Similarly, SIRT2 knockdown by siRNA or HSP70 overexpression reduced the neurotoxic effects of α-synuclein. Together, these findings suggest that both zinc-dependent HDACs as well as SIRT2 are potential targets for therapeutic intervention in PD.

Alzheimer's disease (AD)

AD is the fourth most common cause of death in the USA and affects ~50% of the population over 85 years of age in industrialized countries. Clinically, the disease is characterized by progressive memory loss and personality changes, ultimately leading to dementia. Its neuropathological hallmarks include accumulation of extracellular β-amyloid (Aβ), an aberrant processing product of normal APP, and intracellular neurofibrillary tangles resulting from hyperphosphorylation of Tau protein. Many transgenic mouse models of AD have been generated; these have dramatically advanced our understanding of the pathophysiological mechanisms of AD and of potential strategies to combat the progression of this disease.

Valproic acid, like lithium, appears to decrease Aβ production in HEK293 cells expressing Swedish APP_{751} as well as in the CNS of PDAPP (APP_{V717F}) AD transgenic mice [97]. More recently, Tsai and colleagues studied the role of chromatin remodeling in learning and memory in CK-p25 (CDK5 activator) transgenic mice in which the expression of p25, a protein implicated in various neurodegenerative conditions, can be switched on and off conditionally [98]. In this elegant study, environmental enrichment caused chromatin modification through increased histone-tail acetylation, and reinstated learning and memory even after significant neurodegeneration had taken place in these CK-p25 mice. Moreover, treatment with sodium butyrate markedly improved associative and spatial learning. A recent study from the same group demonstrated that HDAC1 inactivation by p25 is part of the mechanism underlying the ability of p25 to elicit double-strand DNA breaks that precede neurotoxicity [99]. However, the mechanisms underlying p25-mediated HDAC1 inactivation are presently undefined and deserve further investigation. A follow-up study by Guan *et al.* convincingly demonstrated that mice overexpressing HDAC2, but not HDAC1, exhibit decreased dendritic spine density, synaptic number and synaptic plasticity, and impaired memory formation [100]. Conversely, *Hdac2* knockout mice show memory improvement. Further, HDAC2 regulates synaptic formation and plasticity in the mouse hippocampus and binds to promoters of a spectrum of genes involved in neuronal activity, synaptic formation and plasticity. The memory impairment in mice overexpressing HDAC2 was ameliorated by treatment with the HDAC inhibitor vorinostat. These findings underscore the involvement of chromatin modification by HDAC2 in regulating synaptic plasticity and memory formation. The role of histone-tail acetylation in these processes is also supported by several independent studies showing that the HAT activity of CBP is essential for long-term potentiation (LTP) and long-term memory [101].

In the Tg2576 mouse model of AD, daily injections of 4-phenylbutyrate reversed spatial memory deficits by normalizing Tau hyperphosphorylation in the hippocampus but without affecting Aβ levels [102]. 4-phenylbutyrate treatment also ameliorated the dramatic loss of histone H4 acetylation in the cortex and promoted GluR1, PSD95 and MAP2 expression, suggesting that the underlying neuroprotective mechanisms involve normalization of transcriptional dysfunction. In the APP23 transgenic mouse model of AD, daily injections with a relatively low dose of valproic acid (30 mg/kg, i.p.) robustly reduced Aβ plaque number and improved memory deficits when administered early (starting at seven months) [103]. These effects of valproic acid were attributed to inhibition of GSK-3β-mediated γ-secretase cleavage of APP. However, the involvement of HDAC inhibition by valproic acid in these neuroprotective effects was not rigorously examined under the experimental conditions employed. In another study, treatment of the triple transgenic mouse model of AD with nicotinamide, a class III HDAC inhibitor, prevented memory impairments and decreased Tau pathology but without affecting Aβ load or production [20]. Interestingly, nicotinamide treatment also induced a chronic, but low-level, increase in endogenous p25, which surprisingly was linked to improved learning and memory. Although nicotinamide could exhibit its beneficial effects through both sirtuin-dependent and independent mechanisms, these results suggest that class III HDACs are involved in the pathology of AD, and that oral nicotinamide potentially might prove useful as a treatment for this disease.

Conclusions and future directions

Accumulating evidence supports the notion that histone hypoacetylation and transcriptional dysfunction are involved in a large number of neurodegenerative conditions *in vivo* and *in vitro*. In most cases treatment with class I and II HDAC inhibitors normalizes these deficiencies and protects against neurodegeneration. Multiple genes have been identified that are regulated by HDAC inhibition and are involved in neuroprotection and neurotrophicity (Figure 2). HDAC inhibition was found to induce neurotrophin expression not only in

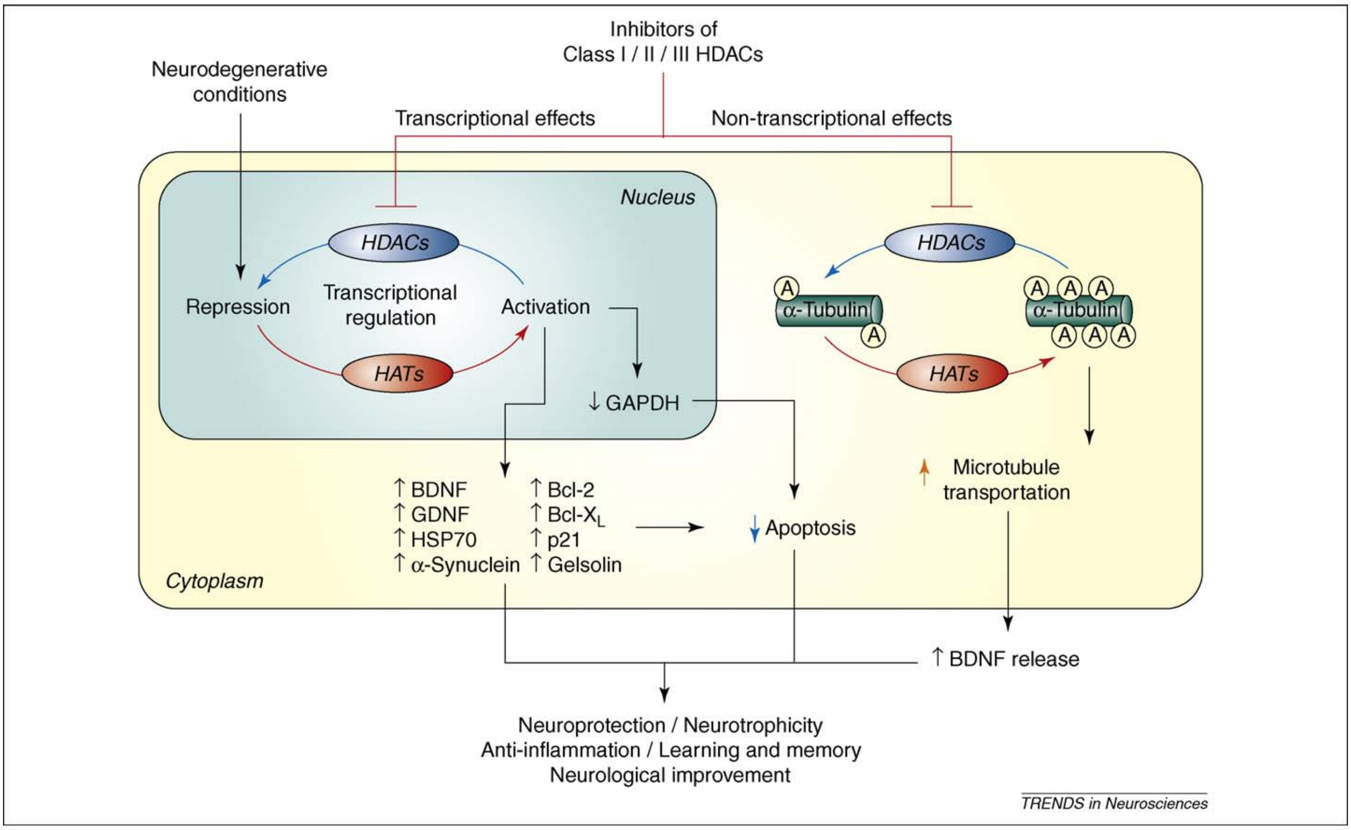

Inhibitors of
Class I / II / III HDACs
Neurodegenerative conditions
Transcriptional effects
Non-transcriptional effects
Nucleus
HDACs
Repression
Transcriptional regulation
Activation
HATs
↓ GAPDH
A
α-Tubulin
A
HDACs
A A A
α-Tubulin
A A A
HATs
Microtubule transportation
↑ BDNF
↑ GDNF
↑ HSP70
↑ α-Synuclein
↑ Bcl-2
↑ Bcl-X$_L$
↑ p21
↑ Gelsolin
↓ Apoptosis
Cytoplasm
↑ BDNF release
Neuroprotection / Neurotrophicity
Anti-inflammation / Learning and memory
Neurological improvement
TRENDS in Neurosciences

Figure 2. Actions of HDAC inhibitors in neurodegenerative conditions. A large number of neurodegenerative conditions *in vivo* and *in vitro* involve functional imbalance in HATs and HDACs, resulting in histone hypoacetylation and transcriptional dysfunction. Treatment with class I, II and, more recently, class III HDAC inhibitors restores these deficiencies. These effects appear to be mediated by multiple HDAC-regulated gene products including, among others, BDNF, GDNF, HSP70, α-synuclein, Bcl-2, Bcl-X$_L$, p21, and gelsolin. Non-transcriptional effects of HDAC inhibitors, including hyperacetylation and stabilization of microtubule proteins, have also been shown in many models of neurodegenerative disease. Studies suggest that HDAC inhibitors have neuroprotective, neurotrophic, and anti-inflammatory effects, and can also improve neurological performance and learning/memory in various neurodegenerative conditions. Bcl-2, B-cell lymphoma 2; BDNF, brain-derived neurotrophic factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GDNF, glial cell line-derived neurotrophic factor; HAT, histone acetyltransferase; HDAC, histone deacetylase; HSP70, heat shock protein 70.

neurons but also in astrocytes, suggesting that glia are also an important target for therapeutic intervention. As noted above, class I and II inhibitors suppress neuroinflammation by inhibiting microglial activation in cultured cells and in stroke models. This raises the possibility that HDAC inhibitors may be developed as anti-inflammatory drugs in the treatment of brain disorders. This is an important area for future research because at present there is no clinical drug that robustly mitigates neuroinflammation in the brain. In addition to transcriptional regulation, non-transcriptional events, such as improvement of microtubule stability via enhanced acetylation, have been implicated in the neuroprotective effects of HDAC inhibition in a number of disease models. HAT-mediated acetylation of HTT is an important mechanism for removing accumulated toxic protein in the HD model. It remains to be investigated whether this is a general mechanism for the selective targeting of proteins for lysosomal degradation and clearance, and whether this is a regulatory target of HDAC inhibitors.

In the diverse animal models of neurodegenerative diseases discussed above, treatments with broad-spectrum, pan-inhibitors of class I and II HDACs demonstrate various degrees of effectiveness in combating neuronal cell death and improving neurological outcome (Table 2). Emerging evidence also suggests that blocking class III HDACs can be neuroprotective in HD, PD, and AD models. In some cases (e.g. HD, PD, SMA, and possibly stroke models), the principal HDAC isoforms involved in the pathology and potential treatment of these diseases have been identified. Paradoxically, in rare cases, the activity rather than the inhibition of certain HDAC isoforms can have neuroprotective effects; these include HDAC1 in the AD model, and SIRT1 in both AD and ALS models [104]. Identification of specific genes whose expression is regulated by these HDACs and that are crucial for the pathological conditions may shed light on the mechanisms underlying these paradoxical effects.

A few isoform-specific HDAC inhibitors are now available. Additional HDAC inhibitors, including both isoform-specific and nonspecific drugs, need to be developed. BBB permeability and cytotoxic profiles of existing HDAC inhibitors require more critical evaluation. The clinical toxicity and side-effects of HDAC inhibitors in cancer treatment have been well-documented [105]. The adverse effects of HDAC inhibitors were often detected after relatively short-term therapy in cancer patients, and might be exacerbated with the longer-term treatment required in patients with neurodegenerative diseases. It seems worthwhile to explore whether the reduced neurotoxicity and beneficial effects of 'pulse treatment' with HDAC inhibitors found in an *in vitro* experimental setting [25] can be replicated in the *in vivo* preclinical models or clinical studies. HDAC isoform-specific drugs would be anticipated to have fewer adverse effects and be better tolerated. However, it is presently unknown whether isoform-specific, or pan-HDAC inhibitors, would be more effective in treating a given neurodegenerative disease. There are

Table 2. Effects of treatment with HDAC inhibitors in models of neurodegenerative diseases

Disease model	Histone hypo-acetylation/ transcriptional dysfunction	Microtubule dysfunction	HDAC inhibitors examined	Beneficial effects after treatment	References
Stroke	Yes	Yes	Valproic acid, vorinostat, sodium butyrate, TSA, 4-phenylbutyrate	Restored histone hypoacetylation and transcriptional dysfunction; enhanced neurogenesis; decreased infarct volume, neuroinflammation and neurological deficits	[41–45,47–49]
Huntington's disease (HD)	Yes	Yes	Vorinostat, sodium butyrate, 4-phenylbutyrate, TSA, HDACi 4b, nicotinamide	Restored histone hypoacetylation and transcriptional dysfunction; normalized striatal atrophy and degeneration; increased BDNF vesicular transport; improved motor performance and survival	[54–56,58–62,66]
Amyotrophic lateral sclerosis (ALS)	Yes	?	4-Phenylbutyrate, valproic acid, 4-phenylbutyrate+antioxidant, valproic acid+lithium, 4-phenylbutyrate+riluzole	Restored histone hypoacetylation and CBP loss; suppressed motor neuronal death; improved motor function and survival	[69–71,73–76]
Spinal muscular atrophy (SMA)	Yes	?	Sodium butyrate, 4-phenylbutyrate, valproic acid, vorinostat, TSA, romidepsin (FK-228)	Increased SMN_2 expression; induced Bcl-2, Bcl-X_L and BDNF; suppressed spinal motor neuronal degeneration and muscle atrophy; prolonged life span	[77–84,86–93]
Parkinson's disease (PD)	Yes	Yes	Valproic acid, sodium butyrate, TSA, vorinostat, AGK2	Increased GDNF and BDNF expression; reduced neuroinflammation and dopaminergic neuronal death; increased acetylation of α-tubulin	[33,36,37,94,96]
Alzheimer's disease (AD)	Yes	Yes	Valproic acid, sodium butyrate, 4-phenylbutyrate, nicotinamide, vorinostat	Restored histone hypoacetylation; increased synaptic plasticity; decreased Aβ production and *Tau* hyperphosphorylation; reinstated learning and memory; reversed spatial memory deficits	[20,97–100,102,103]

indications that combined treatment of an HDAC inhibitor with another neuroprotective drug can have additive or even synergistic effects, suggesting that combinatorial approaches should be pursued. Despite the fact that research in this area is still in its infancy, HDAC inhibition is a promising new avenue for therapeutic intervention in neurodegenerative disorders. The development of potent and effective HDAC inhibitors with excellent BBB permeability, reduced cytotoxicity, and fewer undesirable side-effects remains a major challenge; many crucial issues will need to be addressed before these promising drugs can be effectively used in the clinic.

Acknowledgements

This work was supported by the Intramural Research Program of the National Institute of Mental Health (NIMH) at the National Institutes of Health (NIH). We thank Jau-Shyong Hong and his colleagues at the National Institute of Environmental Health Sciences (NIEHS), NIH, for their collaboration. The critical comments of Li-Kai Tsai and Zhifei Wang (Molecular Neurobiology Section, NIMH, NIH) and the editorial assistance of Peter Leeds (NIMH) and Ioline Henter (NIMH) are appreciated. We also wish to apologize to investigators whose related papers are not cited in this review due to space constraints.

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